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Comprehensive two-dimensional gas chromatography—quadrupole mass spectrometric analysis of drugs

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Abstract

The use of comprehensive two-dimensional gas chromatography coupled to quadrupole mass spectrometry (GC × GC–qMS) for drug screening is investigated with 77 underivatised drug standards in methanolic solution. The GC × GC–qMS setup involved a reduced mass scan range of 42–235 u and minimum quadrupole sampling time to achieve quadrupole scanning frequency of 19.36 Hz. Only 26% of the drugs investigated gave fair-to-acceptable library matches with full mass scan range commercial libraries. The creation of a new "truncated" library based on the mass spectra of the drug standards in the applied mass scan range of 42–235 u extends the feasibility of the currently adopted GC × GC–qMS approach to higher molecular weight compounds and is investigated with blank blood spiked with drug standards. With the new library, 75% of the drugs yielded matches of at least 90%. The time-of-flight mass spectrometer (TOFMS) is expected to address the limitations of the present GC × GC–qMS setup and a brief comparison between GC × GC–qMS and GC × GC–TOFMS is also provided in this study.
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Keywords: Drug screening; Multi-dimensional chromatography; Mass spectrometry library

1. Introduction

The area of toxicological (clinical; forensic; doping control) analyses is vast with challenges specific to the target analytes and the matrices from which they are extracted. Numerous instrumental techniques address these challenges and among them, GC with quadrupole mass spectrometry (qMS) detection plays an important role in the forensic laboratory. GC–MS is a widely accepted and mandated chemical test for the confirmation of (presumed) positive samples [1–3] although its versatility also allows use for screening and/or quantitative purposes.

Comprehensive two-dimensional GC (GC × GC) is a novel approach for the analyses of complex samples and over the past decade, its applicability has been demonstrated for a varied range of samples types such as natural products [4,5], essential oils [6] and environmental toxicants (e.g. pesticides) [7]. Reviews of GC × GC principles have been presented elsewhere [8,9].

The implementation of GC × GC requires fast detector acquisition rates to provide sufficient data density for accurate definition of the narrow ^{2}D peaks, which are often reported to be less than about 100 ms wide. This is strongly emphasized in GC × GC quantitative studies where slow data rates can result in inaccurate peak measurement. While most reported analyses use flame ionisation detectors (FID) with data acquisition rates of up to 200 Hz, attempts have been made by Frysinger and Gaines [10], Shellie and Marriott [11] as well as Debonneville and Chaintreau [12] to use qMS detection for GC × GC (GC × GC–qMS). With the structural-informing ability of qMS, it is possible to use this to support GC × GC separations with mass spectrometric identification, and perform semi-quantitative analysis. These studies implemented the three-dimensional GC × GC–qMS technique using different strategies, recognising that the main experimental challenge in GC × GC–qMS stems from the slow qMS scan rate. Frysinger and Gaines [10] slowed down the GC × GC separation, by increasing the ^{1}D column length to obtain an average ^{2}D peak width of 1 s, so as to match the qMS scan rate of 2.43 Hz. This incurred a total analysis time of about 7 h. Shellie and Marriott [11] adopted a reduced mass scan

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Table 1
Composition of standards A–D, their molecular masses, matches to full mass spectral and truncated libraries, as well as total retention times for GC–MS and GC × GC–qMS

Std. mix	No.	Drug	Molecular mass (g/mol)	Total retention time (min)		Library match (%)		
				GC–MS	GC × GC–qMS[a]	NIST or Wiley library		User-created library
						GC–MS	GC × GC–qMS	GC–MS
A	1	Methamphetamine[b]	149.1	11.67	11.43	83	90	83
	2	Amphetamine[b]	135.2	ND	ND	–	–	–
	3	Methylenedioxyamphetamine (MDA)[b]	179.2	ND	ND	–	–	–
	4	Methylenedioxymethamphetamine (MDMA)[b]	193.1	20.84	20.77	91	90	94
	5	Amylobarbitone (2 μg/mL)[b]	226.1	25.69	25.51	91	91	91
	6	Pentobarbitone (2 μg/mL)[b]	226.1	26.28	26.11	91	96	91
	7	Pethidine	247.2	26.05	25.97	99	–	90
	8	Caffeine (2 μg/mL)[b]	194.1	28.28	28.20	98	96	98
	9	Lignocaine[b]	234.2	28.77	28.71	91	90	95
	10	Doxylamine	270.2	29.63	29.58	90	91	91
	11	Methadone (0.5 μg/mL)	309.2	34.05	33.98	96	83	91
	12	Amitriptyline	277.2	35.39	35.32	90	90	91
	13	Nortriptyline	263.2	35.82	35.72	97	91	91
	14	Moclobemide	268.1	36.78	36.66	94	78	91
	15	Dothiepin	295.1	38.96	38.86	91	78	91
	16	Clomipramine	314.2	39.35	39.25	99	–	95
	17	Diazepam	284.1	40.13	40.00	99	–	93
	18	Nordiazepam	270.1	41.53	41.41	99	–	95
	19	Temazepam	300.1	42.67	42.54	99	–	93
	20	Haloperidol (0.5 μg/mL)	375.1	48.16	48.07	99	–	83
	21	Thioridazine	454.3	50.41	50.40	98	–	90
	22	Verapamil	370.2	50.53	50.40	94	–	95
B	23	Phentermine[b]	149.1	10.71	10.69	90	78	90
	24	Fenfluramine[b]	231.1	12.54	12.42	72	90	80
	25	Nicotine[b]	162.1	15.76	15.77	95	95	97
	26	Pseudoephedrine[b]	165.2	ND	ND	–	–	–
	27	Cotinine[b]	176.1	25.03	24.99	97	97	95
	28	Diphenhydramine	255.2	28.64	28.58	78	91	91
	29	Phencyclidine	243.2	29.30	29.24	99	–	91
	30	Tramadol (0.5 μg/mL)	263.2	30.46	30.44	95	–	90
	31	Venlafaxine	277.2	33.23	33.18	64	–	90
	32	Propoxyphene	339.22	34.77	34.71	87	–	90
	33	Cocaine	303.2	35.63	35.59	99	–	95
	34	Imipramine	281.2	35.93	35.85	99	–	95
	35	Desipramine	266.2	36.46	36.39	99	–	93
	36	Promethazine	284.1	37.03	36.92	97	–	91
	37	Sertraline	305.1	38.68	38.59	99	–	90
	38	Citalopram (0.5 μg/mL)[c]	324.4	39.26	39.18	97	–	95
	39	Hydrocodone	299.2	40.39	40.34	99	–	91
	40	Oxycodone	315.2	41.67	41.61	99	–	–
	41	Nifedipine	346.3	41.98	ND	96	–	–
	42	Flunitrazepam (0.1 μg/mL)	313.1	42.97	ND	99	–	–
	43	7-Aminoflunitrazepam (0.5 μg/mL)[c]	283.1	43.47	43.36	93	–	70
	44	7-Aminonitrazepam (0.5 μg/mL)	251.1	45.37	45.30	98	–	83
	45	Nitrazepam (0.2 μg/mL)	281.1	45.69	ND	99	–	–
	46	7-Aminoclonazepam (0.5 μg/mL)	285.7	46.81	46.71	70	–	–
	47	Diltiazem[c]	414.2	47.73	47.62	98	–	91

Table 1 (*Continued*)

Std. mix	No.	Drug	Molecular mass (g/mol)	Total retention time (min)		Library match (%)		
				GC–MS	GC × GC–qMS[a]	NIST or Wiley library		User-created library
						GC–MS	GC × GC–qMS	GC–MS
	48	Triazolam (0.2 μg/mL)	342.0	49.53	ND	91	ND	–
	49	Strychnine (0.5 μg/mL)	334.2	51.41	51.32	99	–	93
C	50	Ephedrine[b]	165.2	ND	ND	–	–	–
	51	Fluoxetine	309.1	28.39	28.37	96	90	90
	52	Chlorpheniramine	274.1	31.66	31.59	91	91	70
	53	Propranolol	259.2	ND	ND	–	–	–
	54	Mianserin	264.2	35.84	35.80	99	–	95
	55	Doxepin	279.2	36.08	35.99	96	78	91
	56	Benzhexol	301.2	36.42	36.32	92	78	98
	57	Bupivacaine	288.2	36.82	36.72	90	90	91
	58	Benztropine	307.2	37.64	37.59	91	62	93
	59	Codeine	299.2	39.25	39.20	99	–	91
	60	Chlorpromazine	318.1	40.96	40.87	99	–	91
	61	Paroxetine	329.1	42.38	42.34	99	–	94
	62	Metoclopramide	299.1	42.89	42.81	93	83	91
	63	Trifluperazine	407.2	43.54	43.46	99	–	94
	64	Olanzapine (0.5 μg/mL)	312.4	44.88	44.82	99	–	58
	65	Quinine (2 μg/mL)	324.2	46.00	45.88	91	86	90
	66	Prochlorperazine	373.1	48.35	48.28	99	–	93
	67	Pholcodine	398.2	51.19	51.11	93	53	91
	68	Quetiapine	383.5	ND	ND	–	–	–
D	69	Paracetamol (20 μg/mL)[b]	151.1	25.50	25.66	95	94	–
	70	Fluvoxamine	318.2	28.86	28.81	81	–	–
	71	Mirtazapine (0.5 μg/mL)	265.2	36.68	36.66	99	–	76
	72	Zopiclone (0.5 μg/mL)	388.8	50.32	ND	94	–	–
	73	Carbamazepine	236.1	38.18	38.02	99	–	91
	74	Phenytoin (20 μg/mL)	252.1	38.89	38.68	98	–	94
	75	Midazolam (0.5 μg/mL)	325.1	42.61	42.54	99	–	95
	76	Alprazolam (0.5 μg/mL)	308.1	48.23	48.16	99	–	95
	77	Zolpidem (0.5 μg/mL)	307.2	45.80	ND	99	–	–

ND: not detected.

[a] ^1t_R of the largest pulse of the peak envelope is reported.

[b] Drugs whose molecular weights fall within the GC × GC–qMS mass scan range of 42–235 u.

[c] Drugs matched by the in-house and a commercial toxicology (PMW_Tox2) mass spectral library at the Victorian Institute of Forensic Medicine.

range of 40–228 = 188 u with minimum qMS sampling time, achieving a spectral acquisition rate of 20 Hz. Average ^{2}D peak widths were about 100 ms, with total analysis time of 66.7 min and library match qualities of over 90% for most of the 65 components identified in a geranium essential oil sample; most components had molar masses below the upper scan mass range. Recently, (semi)-quantitative analysis of 24 allergens in perfume samples was performed in the single-ion monitoring (SIM) mode using GC × GC–qMS, by Debonneville and Chaintreau [12]. With this set up, qMS sampling time of 10 ms and a data acquisition rate of 30.7 Hz were used for measuring peak widths as low as 54 ms for limonene.

In comparison to qMS, time-of-flight mass spectrometric detection (TOFMS) is capable of presenting mass spectra at up to 500 Hz. With TOFMS, the problem of mass spectral distortion as a result of concentration changes in the ion source is eliminated [13]. The TOFMS's deconvolution ability can also be used to resolve peaks in the mass spectral domain by mathematically generating "clean" mass spectra. These TOFMS features complement the superior separation and increased peak capacity in GC × GC. Several works have reported GC × GC coupled to TOFMS for various analyses [14–17].

Despite the strengths of GC × GC–TOFMS, cost (purchase and maintenance) may be an obstacle for adopting the technique as a routine analytical method. The large data files generated by GC × GC–TOFMS at data acquisition rates of 50 Hz (or higher) demand long data-processing time and large hard disk memory space for data handling [15,18,19]. Automated data-processing of a GC × GC–TOFMS analytical run of cigarette smoke took approximately 7 h [14]. Clearly, the large data files and time-consuming data handling processes associated with GC × GC–TOFMS are impractical for a routine laboratory with high sample throughput. Quadrupole mass spectrometers are widely available in most laboratories, and so GC × GC–qMS is an attractive alternative if acceptable MS data, or suitable

library-searchable data, can be produced within the scanning constraints. Following Shellie et al. [8], GC × GC–qMS is used here as a screening procedure using four standard stock mixtures, which comprised underivatised drugs that are likely to be encountered in a forensic toxicological screen, to examine the scope protocols required for GC × GC–qMS for drugs analysis. This is a comparative study to another which employed GC × GC with TOFMS detection [20] for drug screening.

2. Materials and methods

2.1. Chemicals, standards, biological materials and extraction procedures

Drugs were obtained as pure reference standards from various sources, including the curator of standards at the Australian Government Analytical Laboratories; Sigma–Aldrich Australia and from the forensic standards officer of the Forensic Toxicology Laboratory (Division of Analytical Lab., Sydney, Australia). Drug stock solutions (standards A–D) were prepared in HPLC grade methanol (BDH Lab. Supplies, Poole, England). Standards A–D were each composed of a mixture of underivatised drugs and their compositions are listed in Table 1. Final concentrations of each drug in the drug stock solutions were 1 mg/L unless otherwise stated in parenthesis in Table 1. Expired whole blood from the local blood bank (Victoria, Australia) was used as drug-free blood, after the blood was tested by routine drug screening methods, e.g. ELISA, GC with both MS and nitrogen–phosphorus detection [21], and HPLC [22] by the Victorian Institute of Forensic Medicine (VIFM). All sample preparation was performed at VIFM.

For spiked blood standards, 25 μL of drug stock solution (standards A–D), 500 μL of 2 M Trizma base (Sigma Chemical Co., St. Louis, Missouri, USA) at pH 9.2, and 500 μL drug-free blood were successively added to a silanised glass extraction tube. Extraction was performed by adding 8 mL of butyl chloride (HPLC grade, BDH Lab. Supplies, Poole, England) to the tube, with mixing on a rotation wheel for 30 min. The tubes were centrifuged (2350 × *g*) for 5 min and the blood layer frozen in an ethanol bath (−30 °C). The butyl chloride layer was transferred to a clean silanised glass extraction tube and evaporated to dryness in a vacuum sample concentrator (Savant Industries, Australia). The residue was reconstituted with 100 μL HPLC grade methanol and transferred to an autosampler GC vial containing a micro glass insert.

2.2. Instrumental

2.2.1. GC × GC–quadrupole mass spectrometry (GC × GC–qMS) and GC × GC–TOFMS

GC × GC–qMS was carried out on an Agilent Technologies 6890 model GC system (Agilent Technologies, Burwood, Australia), which was retrofitted with a longitudinally modulated cryogenic system (LMCS; Chromatography Concepts, Doncaster, Australia). The instrument was equipped with a 5973 mass selective detector, a model 6873 auto sampler and Chemstation software. The column set used consisted of a primary capillary column of dimensions 30 m × 0.25 mm i.d. × 0.25 μm film thickness BPX5 phase (5% phenyl equivalent) serially coupled with a second capillary column of dimensions 0.8 m × 0.1 mm i.d. × 0.2 μm film thickness BPX50 phase (50% phenyl equivalent). Both columns were from SGE International (Ringwood, Australia). Both the CO_2 supply and LMCS modulation (frequency of 0.25 Hz) were started at 4 min. The thermostatically controlled cryogenic trap was maintained at about −30 °C for the duration of each analysis.

Temperature programme conditions were as follows: initial temperature of 70 °C for 0.5 min, programmed at 5 °C/min to 320 °C; and held for 5 min at 320 °C (55.50 min total run). The injector temperature was 250 °C with an injection volume of 0.2 μL in the splitless mode. Constant He carrier gas pressure, at an initial flow rate of 1 mL/min, was applied throughout the whole analysis.

The MS transfer line temperature was 280 °C, MS detector voltage 1.8 kV, and a reduced mass scan range of 42–235 u was used to give a data acquisition rate of 19.36 Hz. Spectra were matched with the NIST98 and Wiley275 MS libraries using the ChemStation software.

For GC × GC–TOFMS, a LECO Corporation (LECO, St Joseph, MI) Pegasus III instrument with an Agilent 6890GC, was fitted with an LMCS unit as described above. The column set comprised a 30 m × 0.25 mm i.d. × 0.25 μm film thickness HP-5MS phase (5% phenyl equivalent; Agilent Technologies, Burwood, Australia) coupled with a second capillary column of dimensions 1.0 m × 0.1 mm i.d. × 0.1 μm film thickness BPX50 phase. The temperature program was 70 °C for 0.2 min, programmed at 5 °C/min to 320 °C; and held for 10 min at 320 °C, with constant He carrier gas flow rate of 1.5 mL/min. A data rate of 50 Hz and mass scan range of 40–900 u was employed.

2.2.2. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses were carried out using the same setup as (A). Conventional GC–MS was effected by not activating the modulator, i.e. without provision of CO_2. All conditions were the same as (A), except that a larger injection volume of 1 μL was employed in the splitless mode, and a mass scan range of 40–500 u was used at a scan rate of 3.18 Hz.

2.3. Data analysis and presentation

Data acquisition by the Agilent ChemStation software was used which allows raw data to be exported as a comma separated value file in ASCII format. The data transformation process for presenting the GC × GC re-

Table 2
Library search results, hit list rankings, base peak and molecular weight of selected drugs analysed by reduced mass scan range GC–MS (42–235 u) and by the described GC × GC–qMS analysis

Drug	GC–MS[a]				GC × GC–qMS[b]			
	Hit list rankings	Match quality (%)	Base peak (u)	M_R (g/mol)	Hit list rankings	Match quality (%)	Base peak (u)	M_R (g/mol)
Methamphetamine	Methamphetamine	83	58	149.1	Methamphetamine	83	58	149.1
	Phentermine	74	58	149.1	Phentermine	47	58	149.1
	MDMA	9	58	149.1	Tramadol	9	58	263.2
MDMA	MDMA	94	58	193.1	MDMA	94	58	193.1
	Diltiazem	9	58	414.2	Doxylamine	9	58	388.2
	Doxylamine	9	58	388.2	Methamphetamine	9	58	149.1
Tramadol	Tramadol	90	58	263.2	Tramadol	91	58	263.2
	Propoxyphene	39	58	339.2	Citalopram	39	58	324.4
	Venlafaxine	39	58	277.2	Venlafaxine	39	58	277.2
	Diphenhydramine	39	58	255.2	Propoxyphene	9	58	339.2
Dothiepin	Dothiepin	91	58	295.1	Dothiepin	91	58	295.1
	Amitriptyline	56	58	277.2	Propoxyphene	38	58	339.2
	Doxepin	38	58	279.2	Amitriptyline	23	58	277.2
Thioridazine	Thioridazine	90	98	370.2	Thioridazine	91	98	370.2
	Benzhexol	12	98	301.2	Benzhexol	9	98	301.2
Chlorpromazine	Chlorpromazine	91	58	318.1	Chlorpromazine	91	58	318.1
	Propoxyphene	37	58	339.2	Propoxyphene	37	58	339.2
	Citalopram	9	58	324.4	Citalopram	25	58	324.4
Benzhexol	Benzhexol	98	98	301.2	Benzhexol	91	98	301.2
	Cotinine	1	98	176.1	Thioridazine	2	98	370.2
	Thioridazine	1	98	370.2				
Moclobemide	Moclobemide	91	100	268.1	Moclobemide	91	100	268.1
Doxylamine	Doxylamine	91	58	388.2	Doxylamine	91	58	388.2
	Diphenhydramine	32	58	255.2	Citalopram	12	58	324.4
	Citalopram	9	58	324.4	Diphenhydramine	12	58	255.2
					MDMA	9	58	193.1

[a] Spiked blood standards.
[b] Methanolic drug standards.

sults in 2D contour plots has been described elsewhere [23].

2.4. Applicability of a user-created MS library in reduced mass scan range (42–235 u)

To test the effectiveness of the "truncated" library, spiked blood standards (see Section 2.1) were analysed by GC–MS before they were compared with the user-created library (match results are presented in Table 1). The conditions used for GC–MS analysis were the same as described in Section 2.2.2, except that the mass scan range was 42–235 u scanned at 6.97 Hz. Description of generation of the truncated library is provided in Section 3.4 below.

The PBM search algorithm of the ChemStation software is further tested with a selection of methanolic drug standards and spiked blood standards analysed by GC × GC–qMS and by reduced mass scan range GC–MS, respectively (see previous paragraph). The hit list rankings and match qualities of the drugs are presented in Table 2.

3. Results and discussion

3.1. Protocol

The protocol involved analysing each of the standard mixes by GC–MS, followed by GC × GC–qMS. This facilitates identification of the peaks acquired by the described GC × GC–qMS setup. The composition of the standards and GC conditions (e.g. temperature programming) in both GC–MS and GC × GC–qMS were chosen to minimise peak overlap.

Drugs that were analysed by GC–MS but could not be identified by mass spectral matching with either the NIST or Wiley reference library, were matched by in-house or commercial toxicology library (PMW–Tox2) in the reference forensic laboratory (refer to footnote "c" in Table 1). In cases where no library matches were obtained by GC × GC–qMS due to the reduced mass scan range, ^{1}D retention times (^1t_R) were used for tentative identification.

There was generally good correlation between the ^1t_R for GC–MS and GC × GC–qMS, with differences of about

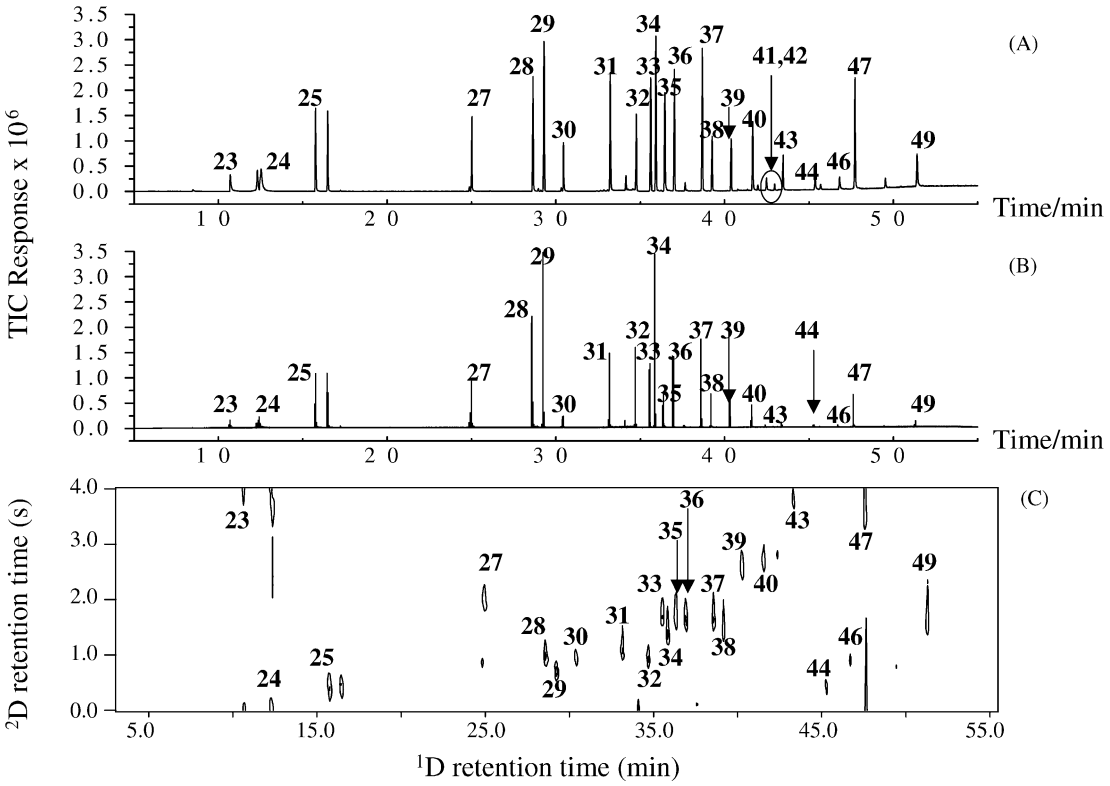

Fig. 1. Chromatograms of standard B by: (A) GC–MS analysis (1 μL injection volume) at 3.18 Hz; (B) GC × GC–qMS analysis (0.2 μL injection volume) at 19.36 Hz; (C) GC × GC–qMS: 2D contour plot. Assignments of the drugs are listed in Table 1. Note the comparable peak responses between (A) and (B) despite the use of reduced injection volume for GC × GC–qMS analysis.

0.1 min shown in Table 1. Due to the small elution time difference of the largest pulses of thioridazine and verapamil in GC × GC–qMS, they were both assigned the same ^1t_R in Table 1 (refer to footnote "a"), even though they have marginally different ^1t_R in GC–MS.

Two-dimensional contour plots were also prepared for all GC × GC–qMS results, although only that of standard mix B is shown in Fig. 1B.

3.2. Mass range selection for GC × GC–qMS

The selection of an appropriate mass range (mass difference of about 188 u) depends primarily on the analytes to be analysed and their spectral fragmentation features. If the experimental aim is to distinguish compounds within a homologue series, such as higher alkanes, then the presence of the molecular ion is pivotal to deducing the identity of the analytes. In this case, the mass range used should incorporate the higher mass ranges of the analytes, e.g. 130–328 u (giving a 198 u mass range) since the lower mass range of ions will lack sufficient spectral specificity to identify the alkane homologues.

For this study, the reduced mass range of 42–235 u was chosen because without derivatisation, the lower mass ions are in greater abundance than the higher mass ions [24]. Hence, unlike homologues (e.g. alkanes), the mass spectra of the drugs have a greater likelihood of spectral fragmentation specificity within the lower mass range of 42–235 u. Provided that a high-resolution mass spectrometer can "screen out" interferences, the use of lower mass ions of high abundance for identification or even quantitation (in selected ion monitoring mode) is viable [25].

In the present study, improved resolution (and sensitivity) is provided by GC × GC in the chromatographic (time) domain. Hence, GC × GC–qMS of thermally stable underivatised drugs in the reduced mass range might be advantageous because the separation selectivity of the ^{2}D column can potentially eliminate lower mass ions from interfering sources. The result will be a "cleaner" spectrum with an enhanced and more distinct fragmentation pattern in the lower mass range (42–235 u).

3.3. GC–MS versus GC × GC–qMS

As a result of the reduced mass range and the minimum quadrupole interscan time used in the GC × GC–qMS implementation, it was expected that library matching would be compromised, especially for drug analytes with high molecular masses. This was confirmed when only 27 of the 77 drugs yielded fair-to-acceptable library matches in GC × GC–qMS (refer to Table 1). Interestingly, high quality library matches of 90% and above were obtained for more than half (i.e. 16) of the 27 drugs identified. These 27 drugs contain diagnostic ions in the applied reduced mass scan range.

Acquisition of phencyclidine under the reduced mass scan range did not give a successful library match when compared with full mass range libraries, evidently due to the lack of the $[M-1]^+$ diagnostic ion (242 u) in its mass spectrum. The comparable base peak ion abundances, with injection volumes of 0.2 and 1 μL for GC × GC–qMS and GC–MS respectively, are proof of the sensitivity gain provided by cryogenic focusing in GC × GC. Clearly, the library search protocol is very sensitive to the highest mass ion cluster (242 and 243 u), and leads to excellent matches for phencyclidine (i.e. 99%) for the full mass scan range GC–MS analysis.

In some cases, higher match qualities were obtained from GC × GC–qMS than that of GC–MS (for pentobarbitone, fenfluramine and diphenhydramine). This observation is likely to be a direct consequence of GC × GC superior separation that introduces more-pure solutes to the mass spectrometer. Some drugs that are known for their poor chromatographic properties when underivatised (e.g. amphetamine, MDA, pseudophedrine and propranolol) were also poor candidates in this comparative study. Drugs such as propranolol are known to be especially prone to aging of injection liners and columns which could lead to a progressive loss of sensitivity [21]. Hence, their inclusion in the sample set provides some information about the performance limits of GC–MS and GC × GC–qMS.

Comparison of the chromatographic peaks of GC–MS and GC × GC–qMS experiments in 1D and GC × GC experiments of standard B is shown in Fig. 1A and B. Fig. 1C is an approximate polarity (vertical axis) versus boiling point (horizontal axis) 2D representation of the GC × GC separation based on use of a non-polar/polar column set arrangement. Thus, two properties can now be used to characterise the drugs, and ^{2}D retention time will be based approximately on the drug polarity, provided that no "wraparound" occurs [26].

Based on ^2t_R measurement via targeted mode multidimensional GC with flame ionisation detection [27], nicotine (#25), diphenhydramine (#28), phencylidine (#29) and tramadol (#30) exhibited one wraparound. Thus, diphenhydramine is more strongly retained than phencylidine, tramadol and nicotine in ^{2}D, as illustrated by their apparent ^2t_R in Fig. 1C.

3.4. Creating a new mass spectra (MS) drug library in the reduced mass range (42–235 u)

A solution to overcome the limitations of library matching for GC × GC–qMS results with commercial full mass scan range MS libraries, is to create a new library containing reference data restricted to the reduced mass scan range applied. The new reference library may extend the capability of the described GC × GC–qMS method to identify higher molecular weight analytes, and can be potentially useful for a directed screening approach in the absence of a time-of-flight mass spectrometer (TOFMS).

In this study, the "truncated" library spectra were obtained by the described GC × GC–qMS analysis of the drug standards (refer to Section 2.2 for experimental conditions) for added convenience. Drugs that were not identified by GC–MS and/or GC × GC–qMS analysis (refer to retention time columns in Table 1) were not added into the "truncated" library as references. These drugs comprise of amphetamine, MDA, MDMA, pseudoephedrine, nifedipine, flunitrazepam, 7-aminoclonazepam, ephedrine, propranolol, quetiapine, zopiclone and zolpidem. For closely eluting compounds in ^{1}D and ^{2}D under the described conditions in Section 2.2 (i.e. pethidine and pentobarbitone; thioridazine and verapamil), a separate analysis to ensure better chromatographic resolution was used. Spectral averaging and background subtraction were applied for the drugs investigated, so as to ensure spectral consistency and purity.

Ways to access a truncated library include the masking of the MS references in pre-existing full mass scan range libraries to the required mass range or by re-analysis of the standards under the criteria of the required mass range. The former will be the most attractive if one has access to the NIST Search software programme, since it could be readily tailored to the mass ranges specific for individual application. The latter was the method adopted for creating the new library, although there are a few variations available to achieve this, which are as follows:

1. Normal 1D-GC operation coupled to normal quadrupole or fast quadrupole operation; or
2. Fast GC operation coupled to normal quadrupole or fast quadrupole operation.

Re-analysis of the standards via normal 1D-GC operation (i.e. generally broad peaks) coupled with fast quadrupole operation (e.g. by reduced scan range and minimum interscan time) or that coupled with normal quadrupole operation (e.g. non-minimum interscan time), will deliver the most reproducible mass spectrum since there will be little variation of the mass flux over the mass spectrometric scan duration. Coupling of fast GC operation (i.e. GC × GC or other methods that produce similarly narrow peaks) with fast or normal quadrupole operation is acceptable as long as one is aware of inherent spectral bias that is present.

Multiple entries of the same analyte (obtained by various appropriate approaches) can also be used to increase the probability of positive identification, provided that the multiple entries of other analytes do not push the entry of the correct analyte down the hit list [28]. Visual inspection of the hit list is advised [3,28]. The use of extracted ion chromatograms can also be used to estimate the extent of peak overlapping in GC × GC–qMS for evaluating the mass spectral quality before a spectrum is added as a library reference provided that the ions selected are of sufficient abundance and specificity in the selected mass range. The extraction of identifier ions also extends the current approach of GC × GC–qMS for identification purposes, e.g. by plotting selected ion GC × GC traces, albeit within the limits of the mass range.

3.5. Feasibility of the "truncated" mass spectral library

The applicability of the new library was tested with drugs spiked into drug-free blood and analysed by GC–MS in the reduced mass range of 42–235 u. Indeed, with the new user-created ("truncated") library, many more compounds can be identified compared to the full-mass scan range commercial libraries (see Table 1), with about 67% of the drugs analysed yielding MS similarity matches of at least 90% and above. The exceptions consist of compounds that were undetected either by full-scan GC–MS and/or GC × GC–qMS (possibly due to the much reduced injection volume applied, i.e. 0.2 μL, see Section 2.2.1).

Further testing of the probability-based matching (PBM) search algorithm on the ChemStation software was performed to evaluate the effectiveness of the new library with a selection of drugs. Both methanolic drug standards and that spiked into drug-free blood were used, which were then analysed by GC × GC–qMS and by reduced mass scan range GC–MS, respectively. Most of the drugs in the selection gave incorrect or no library matches with the commercial libraries when analysed by GC × GC–qMS. In addition, the drugs selected have relatively simple fragmentation patterns dominated by one abundant ion in the reduced mass scan range. Drugs that give simple fragmentation patterns and share a common fragment ion (i.e. 58 or 98 u) with many of the other drugs in Table 1 serve as a more rigorous test for the new library's identification capability because they have fewer points of references in their fragmentation patterns (in the reduced mass scan range) and can potentially introduce more difficulty (or ambiguity) in the prefiltering process during mass spectral matching.

Of greater practical impact is that the more simple the spectrum, the greater the rating given to noise, contaminant or matrix ions that appear in the spectrum, during the library matching process (even when the abundance of the interfering ions is relatively low). The presence and absence of diagnostic ions are both important in establishing the fingerprint. Basing identification on single dimension retention time and one major ion (e.g. 58 u) could lead to erroneous conclusions. This would then suggest an alternative MS technique may be required.

Table 2 presents the hit list rankings and match qualities of the selected drugs analysed by both reduced mass scan range GC–MS and by GC × GC–qMS, after comparison of their mass spectra with the references in the new library. Spectral averaging and background subtraction were used for obtaining the match qualities shown in Table 2.

As shown in Table 2, there was little to distinguish between the match qualities of the selected drugs that were analysed by GC–MS and by GC × GC–qMS, except for subtle differences in the percentage ratings obtained and the drugs that occupy the 2nd and 3rd positions of the hit list rankings. The PBM search algorithm was successful in finding all the drugs in Table 2 as the first hit with match qualities of at least 90% for all the drugs under investigation, except for methamphetamine. Although methamphetamine has complete mass spectra within the reduced mass range, it was interesting to note that the new library gave lower matches than with the commercial libraries (refer to Table 1), possibly due to spectral bias in the library or experimental spectrum.

Match qualities and hit list rankings of the selected drugs are useful parameters to provide indication of the new library's ability to distinguish between analytes with very similar fragmentation features. The PBM search algorithm on the ChemStation software identifies the most significant peaks by a combination of their mass-to-charge ratio values and abundances and then comparing with the condensed library spectra [28]. Library formats based on forward and reverse searching were not evaluated.

3.6. Comparison of quadrupole and time-of-flight mass spectrometry detection

Although generation of a specialist truncated library for drugs may be a considerable undertaking in order to permit use of GC × GC–qMS, it should however make access to identification in GC × GC a relatively straightforward process. The data file with fast qMS detection will be larger than that for conventional slower scanning qMS only to the degree that the scan duty cycle is increased. Data processing (e.g. presentation of extracted ion plots) is not a significantly longer task. The strength of GC × GC–qMS is its enhanced separation for qualitative identification of co-eluting species. By maintaining an awareness of the wrap-around (and peak splitting) effects introduced by the GC × GC process, it is also possible to use the technique reliably for semi-quantitative applications, as demonstrated by Debonneville and Chaintreau for GC × GC–qMS (SIM mode) [12]. Provided translation of GC × GC–qMS and GC × GC-FID results can be carried out, then quantitation may be performed by the latter experiment. This may indeed be a more acceptable quantitative experiment since FID response factors may be more readily determined, and more consistent than qMS response factors (notwithstanding the evidentiary benefits of qMS).

The potential occurrence of mass spectral distortion in quadrupole mass scanning means that care must be taken in choosing the mass spectrum for library searching. Fig. 2I(A–C) shows mass spectra taken across different scans of the ^{2}D chlorpheniramine peak analysed by GC × GC–qMS. Note the variability in the relative ion abundances of ions 58 and 203 u, which is caused by the quadrupole scan cycle (from high mass to low mass) and is sensitive to the varying instantaneous mass flux in the ion source over the scan duration. Such observations have also been noted by Vine [1] for GC–MS. Changes in sample concentrations in the (quadrupole) ion source contribute to mass spectral distortion as the ion abundances are clearly different across the chromatographic peak (see Fig. 2I(A–C), note the scales of the vertical axes in (A–C)). Such instances of mass spectral distortion are exacerbated with GC × GC as very narrow peaks of widths as low as 80 ms or less have been reported.

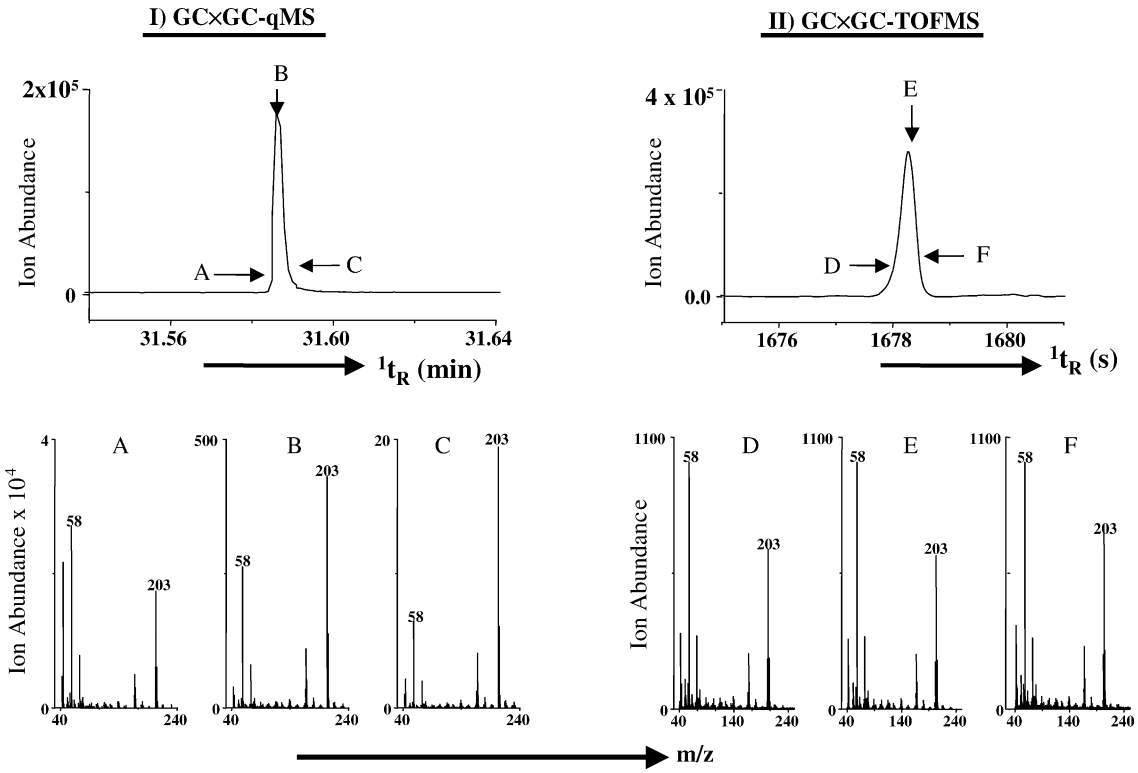

Fig. 2. Comparison of ion abundances of chlorpheniramine at different regions of the chromatographic peak obtained by GC × GC–qMS and GC × GC–TOFMS (I) TIC chromatogram of chlorpheniramine analysed by GC × GC–qMS using 42–235 u acquired at 19.36 Hz. (A) Mass spectrum taken at the front of the ^{2}D peak analysed by GC × GC–qMS. (B) Mass spectrum taken at the apex of the ^{2}D peak analysed by GC × GC–qMS. (C) Mass spectrum taken at the back of the ^{2}D peak analysed by GC × GC–qMS. Note the variation of the relative ion abundances of 58 and 203 u in A–C. (II) TIC chromatogram of chlorpheniramine analysed by GC × GC–TOFMS (LECO Pegasus III, Michigan, USA) using 40–900 u acquired at 50 Hz. (D) Mass spectrum taken at the front of the ^{2}D peak analysed by GC × GC–TOFMS. (E) Mass spectrum taken at the apex of the ^{2}D peak analysed by GC × GC–TOFMS. (F) Mass spectrum taken at the back of the ^{2}D peak analysed by GC × GC–TOFMS.

Contrasting with qMS detection, TOFMS offers speed of data acquisition that is commensurate with quantitative precision of area measurements. Each compound requires specific response factor determination if quantitative measurement is sought. Perhaps the advantages of TOFMS may best be summed up by Fig. 2II(D–F) that shows the mass spectrum taken at the different points across the chlorpheniramine peak analysed by GC × GC–TOFMS. The experiment was performed at 50 Hz at the mass range of 40–900 u (a maximum data presentation rate and mass range of 500 Hz and 1000 u are possible with the LECO Pegasus III TOFMS). The TOFMS system provides for full mass range spectral scanning at maximum scan rate. High data acquisition rates allow accurate definition of the chromatographic profiles that can help to resolve closely eluting peaks, provide effective peak deconvolution, and are of significance in ensuring accurate quantitative peak measurement in GC × GC. TOFMS is also free of mass spectral distortion associated with quadrupole scanning instruments. This is exemplified in the consistency of the relative ion abundances (between ion 58 and 203 u) in the mass spectra of Fig. 2II(D–F) even though the mass spectra were taken at different regions of the narrow GC peak. The GC × GC presentation option provided by the LECO ChromaTOF^{TM} operating software, is an added convenience as it allows direct presentation of TIC and EIC 2D plots without the need for external data conversion and presentation software. Another attractive feature of ChromaTOF^{TM} is its mass spectral deconvolution algorithm that can locate and identify coeluting analytes based on constancy of ion ratios across a GC peak acquired by TOFMS detection. This method is reliable provided that coeluting analytes do not share common "unique" ions. However, data files acquired by TOFMS at high acquisition rates (e.g. ≥50 Hz) are large (much larger than with qMS, even when considering the scan speed differences), and automated detection as well as data presentation is time-consuming. Despite the ability of TOFMS to sample up to 500 Hz, the trade-off between sensitivity (S/N) and high data acquisition rates leads to loss of peak response at high rates.

4. Conclusion

Validation of GC × GC separations with qMS identification can be realised with the present experimental approach employing truncation of reference spectral library. Instru-

mental limitations (scan range; scan speed) of the quadrupole mass spectrometer are due to the nature of the quadrupole duty cycle. The semi-volatile nature and high molecular mass of some of the drugs used, compromise solute identification in GC × GC–qMS when truncated data are acquired, and render full mass range commercial libraries unsuitable. The creation of a reduced mass scan library over the same range as for experimental data offers an analytical solution to the problem of obtaining good mass spectral matches of GC × GC–qMS results if commercial libraries are used, and appears promising using the applied PBM search algorithm of the ChemStation software. A reduced mass range is expected to be most useful where the residual spectrum is sufficiently unique to overcome excluded mass ions (especially the molecular ion). Improved identification should result if retention time data in the two dimensions can be incorporated into the search methodology, and if an expanded "truncated" library is developed.

GC × GC–qMS has the potential for useful problem-solving capabilities in GC × GC analysis. Whilst only 'full-scan' MS acquisition was used here, fast SIM mode in qMS should also be applicable to the GC × GC experiment. Time-of-flight mass spectrometry (TOFMS) is considered the most promising technology for GC × GC, with data acquisition rates of up to 500 Hz at full mass range (0–1000 u). TOFMS also offers mass spectral deconvolution, which increases identification power. Considering the relatively few TOFMS instrument placements (and initial capital expense of TOFMS), the ease with which the qMS instrument can be configured to permit GC × GC operation, and the considerable shorter processing of GC × GC–qMS data, the alternative step of developing a qMS approach that yields GC × GC–qMS interpretation for both identification and quantitative purposes should be an attractive option to many current and potential GC × GC users.

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